

## **Performance Monitoring Protocol (QA/QC) for the AB Sciex API Triple-Stage Quadrupole LC/MS**

### **1 Scope**

This document addresses the performance monitoring (QA/QC) of the AB Sciex Triple-Stage Quadrupole Liquid Chromatograph/Mass Spectrometer (LC/MS) system consisting of an API Triple-Stage Quadrupole MS and a Shimadzu LC. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

### **2 Principle**

The API system is comprised of a Shimadzu LC and an API Triple-Stage Quadrupole (in MS or MS/MS mode). The instrument is configured with an Atmospheric Pressure Ionization (API) source that is capable of both electrospray (ESI) and atmospheric pressure chemical ionization (APCI). Currently, the instrument is primarily used as a Triple-Stage Quadrupole in the ESI mode. However, this protocol can also be used for APCI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

### **3 Equipment/Materials/Reagents**

- a. Instrumentation – API Triple-Stage MS, API Source, Shimadzu LC, and data system with “Analyst” software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Collision Gas & Ion Trap Gas - Nitrogen, 99.999% (high purity or equivalent)
- d. Methanol, HPLC grade or equivalent
- e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- f. Acetonitrile, HPLC grade or equivalent
- g. Formic Acid - ~98% (Fluka or equivalent)
- h. AB Sciex PPG (Polypropylene Glycol) 3000 (included in Part # 401936)
- i. AB Sciex Mass Spectrometer Solution Kit (Part # 401936)
- j. Caffeine (Sigma or equivalent)

- k. Agilent ESI Tuning Mix
- l. Ammonium Acetate, reagent grade
- m. Codeine (Sigma or equivalent)
- n. Brucine (Sigma or equivalent)
- o. Reserpine (Sigma or equivalent)
- p. Volumetric glassware
- q. Infusion Syringe - 10 to 500  $\mu$ L LC syringe (Hamilton or equivalent)

## **4 Standards and Controls**

### **4.1 Testmix**

The stock testmix is prepared by weighing 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. The testmix is further diluted by using 50  $\mu$ L of the stock testmix and diluted to 10-mL volumetric flask with methanol. Store the solution in the refrigerator. It has a shelf-life of three years. The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system.

### **4.2 Calibration Solutions**

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. Using this instrument requires it to be calibrated both in the positive and negative mode. As a result, two calibration solutions are needed to calibrate this instrument.

#### **4.2.1 Preparation of Positive PPG Dilution Solvent**

This solution can be purchased directly from AB Sciex or prepared as follows:

- a. Dissolve 15.4 milligrams of ammonium acetate in 49.9 mL of deionized water.
- b. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.
- c. Mix the two solutions together to make the Positive PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf-life of three years.

#### **4.2.2 Preparation of Negative PPG Dilution Solvent**

This solution can be purchased directly from AB Sciex or prepared as follows:

- a. Dissolve 385.0 milligrams of ammonium acetate in 49.9 mL of deionized water.
- b. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.
- c. Mix the two solutions together to make the Negative PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf-life of three years.

#### **4.2.3 Preparation of Diluted PPG Standard Solution (Positive Mode)**

The Diluted PPG Standard Solution is used to calibrate the Q1 (Quadrupole) and Q3 in the positive mode. The PPG Standard (in Mass Spectrometer Solution Kit) purchased from AB Sciex is diluted with the prepared positive PPG Dilution Solvent (see section 4.2.1) or the one supplied from the purchase kit at a ratio of 1:500 to produce a solution that is  $2.0 \times 10^{-7}\text{M}$ .

- a. Add 1.0 mL of PPG Standard ( $1 \times 10^{-4}\text{M}$ ) to 49.0 mL of the positive PPG Dilution Solvent and mix. This produces a  $2 \times 10^{-6}\text{M}$  solution.
- b. Add 2.0 mL of  $2 \times 10^{-6}\text{M}$  solution to 18.0 mL of the positive PPG Dilution Solvent and mix. This produces a solution that is  $2 \times 10^{-7}\text{M}$ . Store the solution in the refrigerator. It has a shelf-life of three years.

#### **4.2.4 PPG 3000 Standard Solution (Negative Mode)**

The PPG 3000 Standard ( $3.0 \times 10^{-5}\text{M}$ ) is used to calibrate the Q1 (Quadrupole) and Q3 in the negative mode. The PPG 3000 Standard purchased directly from AB Sciex is diluted with the prepared negative PPG Dilution Solvent (see section 4.2.2) or the one supplied from the purchase kit at a ratio of 1:10 to produce a solution that is  $3.0 \times 10^{-5}\text{M}$ .

- a. Add 2.0 mL of PPG 3000 Standard to 18.0 mL of the negative PPG Dilution Solvent and mix. This produces a  $3 \times 10^{-5}\text{M}$  solution. Store the solution in the refrigerator. It has a shelf-life of three years.

### **5 Calibration**

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

#### **5.1 Calibration of Q1 Quadrupole in the Positive Mode**

- a. Load a 250  $\mu\text{L}$  syringe with the Diluted PPG Standard Solution (see section 4.2.3).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10  $\mu\text{L}/\text{minute}$ .

- d. Enter the 'Manual Tuning' window (Refer to API LC/MSMS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q1 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution'.
  - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA (Multi-Channel Averaging) mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

## **5.2 Calibration of Q3 Quadrupole in the Positive Mode**

- a. Load a 250  $\mu$ L syringe with the Diluted PPG Standard Solution (see section 4.2.3).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q3 Pos PPGs.dam' reference file.

- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution'.
  - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

### **5.3 Calibration of Q1 Quadrupole in the Negative Mode**

- a. Load a 250 µL syringe with the PPG 3000 Standard Solution (section 4.2.4).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 µL/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q1 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Neg' is loaded under the dialog box label 'Standard'.

- Q1 and Unit are checked under 'Quad/Resolution' section.
  - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

#### **5.4 Calibration of Q3 Quadrupole in the Negative Mode**

- a. Load a 250  $\mu$ L syringe with the PPG 3000 Standard Solution (section 4.2.4).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q3 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
- Correct tune file is loaded.
  - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution' section.
  - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.

- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

## 5.5 Completion of Calibration

If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

## 6 Sampling or Sample Selection

Not applicable.

## 7 Procedures

### 7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 1GB of free disk space. Do not use if less than 1 GB remain.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 60 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- c. Verify that the system is under vacuum and the ion gauge is reading less than  $6.0 \times 10^{-5}$  torr.

- d. Prepare the instrument for analysis of the testmix. Verify that the instrument has the correct source probe installed (ESI), positive ion mode selected, and centroid data is being collected.
- e. Perform an analysis of the testmix prior to the analysis of samples using parameters listed in the 'Instrumental Conditions' section of this protocol. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'testmix' (or equivalent). Make three 2  $\mu$ L injections of the testmix solution approximately 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the combined ion chromatogram and extracted ions for all components in the testmix.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

## 7.2 As Needed Checks

- a. Replace temperature heaters.
- b. Clean or replace the heated capillary.

## 7.3 Manufacturer's Maintenance Agreement

Calibration and repair will be performed as needed. Under the maintenance agreement with AB Sciex, the manufacturer will conduct an annual preventative maintenance.

## 8 Instrumental Conditions

### 8.1 Testmix (Positive Mode)

#### Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	2 $\mu$ L
Number of Inj:	3

#### Mass Spectrometer

Ionization:	ESI
Scan Mode:	SIM
Source Temp:	600°C
Mass:	95, 300, 395 and 609 m/z

## 8.2 Calibration

### Mass Spectrometer

Ionization: ESI  
Scan Mode: MRM  
Scan Range: Manufacturer's Performance Specifications in the instrument manuals.

## 9 Decision Criteria

### 9.1 Testmix

Verify the results of the testmix. The following ions should be observed in the three testmix injections:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

### 9.2 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. Calibration in the positive mode for Q1 and Q3, confirms the presence of ions m/z 59.1, 175.1, 500.4, 616.5, 906.7, 1080.8 and 1196.9. In addition, verify that the sensitivity and peak width are within range as specified below.

#### **Positive Mode Q1 and Q3**

	Sensitivity (cps) m/z 906.7	Peak width (amu)
Q1	> 1.5e7	0.6 – 0.8
Q3	> 1.3e7	0.6 – 0.8

Calibration in the negative mode for Q1 and Q3, confirms the presence of ions m/z 45.0, 411.3, 585.4, 933.6 and 1223.8. In addition, verify that the sensitivity and peak width are within range as specified below.

#### **Negative Mode Q1 and Q3**

	Sensitivity (cps) m/z 933.6	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8
Q3	> 1.0e7	0.6 – 0.8

## 10 Calculations

Not applicable.

## 11 Measurement Uncertainty

Not applicable.

## 12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

## 13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

## 14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

*FBI Laboratory Safety Manual*.

Rev. #	Issue Date	History
0	03/14/12	New document.
1	09/10/13	Removed Spark Holland Symbiosis LC from Sections 1,2, and 3. Removed Optima Grade Water, Mobile Phase A, and Mobile Phase B from Section 3. Removed 5000 from Sections 5.1.d, 5.2.d, 5.3.d, and 5.4.d. Changed mobile phase in Section 8.1. Added API 6500 System Installation guide and System Operator's manual to Section 14.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Added 'appropriate instrument support personnel' to Sections 5.5 and 7.1 b & f. Updated heading in Section 6. Changed subunit to discipline in Section 8.1. Updated Instrument Operation and Systems Support in Section 14 and header.

**Approval**

Redacted - Signatures on File

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Date: 09/28/2018

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Date: 09/28/2018

**QA Approval**

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